Cytogenetics in the zoo

Chromosome studies on zoo animals have revealed new information about mammalian evolution, and have also helped towards the preservation of species in danger of becoming extinct

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Although the history of evolution is replete with the fossil evidence of extinct animals and plants, what is worrisome about the situation today is the rate at which species are becoming extinct, and the reasons for this process. Something like one mammalian species disappears from the face of the Earth each year. For many of the species whose last few specimens we see depicted in books, the extinction is the direct or indirect result of Man's interference with their habitat; Man's slaughter for his own commercial benefit; Man's whims of fashion, etc.

In the face of these sad happenings, the zoological gardens of the world have gradually changed their attitudes toward the exhibition of animals. While over a hundred years ago their existence was justified exclusively by the desire of the public to look at strange and exotic beasts, in recent years the zoos have more often become the refuge for rare species of animals that are fast disappearing in the wild. Methods of exhibition have drastically changed and zoo directors have become very mindful of the need to breed their stock, and, as with the giant pandas An-An and Chi-Chi, to exchange animals for breeding purposes. At the same time, zoos have assumed a more forward-looking and scientific attitude toward their charges.

Cultures from skin biopsies

Only in the last 10 years or so has it been possible to undertake accurate chromosome studies on mammals with some ease, and during that time my colleagues and I have conducted such studies on zoo animals in several parts of the world. In general, chromosome studies can be most reliably performed on tissue cultures from skin biopsies, either from living or recently dead animals. Of the approximately 3000 mammalian species living today, something like 400 have been studied by this means and some fascinating findings have been made. The objective of such studies is not only to ascertain the number of chromosomes and their structure but rather to understand better the genetic relationship of various animals and to grasp how evolutionary changes in the structure of animals have been accompanied by changes in the chromosomes. Clearly, to know today the chromosome structure—and for that matter the structure of haemoglobin and other micromolecules—of the mammoth, the glyptodon, of eohippus, and many other well-known mammalian ancestors, would enhance our knowledge of evolution considerably. This is particularly the case since, both in Man and many other mammals currently under study, chromosomal rearrangements are very frequently met with. Are they accidentally related to evolutionary events or are they perhaps in some way causally connected?

My interest in these studies began with the unanswered question of why the mule is proverbially sterile. Interspecific hybrid sterility is, of course, well explored in plants, insects, and in some other "lower" animals, but little is yet understood about its occurrence in mammals. In particular we do not understand the reason why some hybrids are sterile, while others, such as cattle x bison crosses, are fertile. It soon became obvious from my study that the donkey and horse have vastly different chromosomes sets. Not only do they differ in the number of their chromosomes (62 and 64 respectively) but their structure is also quite different one from another. When we studied a zebra x donkey hybrid at the Manila Zoo, we found an amazing discrepancy between the number and structure of parental chromosomes which led us to a systematic study of all equine species.

Cooperation between zoos

The findings are graphically represented in Figure 1. It will be seen that most of these animals could never have been studied in the field. A concerted study, with numerous zoos co-operating, was necessary. All animals shown in the table, including those with 66 and those with 32 chromosomes, are known to breed one with another under appropriate circumstances—an amazing fact. Most of the hybrids produced are sterile, but not all, and the exceptions can be well explained by the close relationship of chromosome number and structure.

Perhaps most problematic in this study has been the finding that the Mongolian wild horse, *Equus przewalskii* Poliakov, has a higher chromosome number (66) than all races of the domestic horse (64). We would like to believe that this finding supports the view of some taxonomists that this animal is truly a distinct species—which it is held not to be by the taxonomical "lumpers". This horse is presumably now extinct in the wild, as two recent expeditions have concluded. Only some 140 specimens live scattered through the world's zoos, and it is immediately apparent that this new knowledge should eventually have some impact on the future breeding of this stock. Unfortunately, domestic horses have been crossed with this wild form in zoos and it is known now that such hybrids are fertile. But by carefully eliminating only those hybrids with 65 chromosomes we should be able to ensure the propagation of the ancestral type imported into zoos in 1900.

The horse is perhaps the best explored domestic animal from the evolutionist's point of view. Simpson has marshalled all the evidence in his well-known book *Horses* to show that Equus originally came from North America, from whence it dispersed. In America (South and North) its ancestors became extinct but the descendants migrated over the now discontinuous Behring landbridge, and evolved to become the wild and domestic horses, the donkeys, hemiones and zebras. This dispersal is illustrated in Figure 2 and it will be seen from the chromosome numbers added here that the farthest distant species—the mountain zebra—has the lowest chromosome number. From this evidence and from studies on Felidae and Bovidae (some of which species dispersed into opposite directions) we would like to believe that the more ancestral species have a higher chromosome number. Through fusion of acrocentrics and by other translocational events, species with lower numbers of chromosomes have evolved, but having more and larger matacentric elements. Chromosomal evidence supports this interpretation and it is thus perhaps not surprising then that what looks to be an ancestral beast, the rhinoceros, is found to have the highest diploid number of all mammals studied to date (82, 82 and 84 in the three of five species examined).

An unexplained phenomenon is why in some groups of animals, like the Equidae, such extensive chromosomal rearrangements have taken place, while in others, for instance the Camelidae, no changes occurred in the chromosomal sets. Camels have much the same history of dispersion and speciation as the horse family. Today there are four South American representatives, the vicuna, guanaco, alpaca and llama, and two Asiatic species, the one-humped dromedary and two-humped Bactrian camel. All have 74 similar chromosomes and, on the respective continents, the species interbreed fertilely. Whether intercontinental species crosses in this family would be possible is an interesting question to be explored in the future with the cooperation of zoos.

Abnormalities in mammals

As might be expected, now and then we have found aberrant chromosome patterns in zoo animals. Just as cytogeneticists have found human mosaic individuals, trisomics and chimeras, so we find their abnormalities among other mammals. Most notable in this respect is, of course, the male tricoloured cat, which is now known to be most frequently composed of different genotypes, such as XX/XY and XX/XXY. The same errors are found among display animals. We are currently studying a male raccoon dog that never showed any interest in the opposite sex. The presumed reason for this failure is his XY/XXY mosaicism.

Studies of sex chromosomes have been of direct benefit to the zoos. Thus, while it is virtually impossible to ascertain the sex in the spotted hyena by external examination, chromosome studies show distinct sex chromosome dimorphism, and the female possesses typical Barr bodies in buccal smears as well as drumsticks of her polymorphonuclear leucocytes. Similar means were used to confirm that the giant panda at London is definitely a female, and it is possible now also to "sex" beavers, a difficult task otherwise. It is no longer necessary to keep "pairs" of animals for years in the hope that they may breed, but without actually knowing their sex.

Taxonomy has benefited from the discovery that the aardwolf has a karyotype indistinguishable from the hyenas and quite different from civets. With this finding an old argument of classification can now be regarded as settled, and similar disputes regarding other species, such as the gibbons, have been clarified.

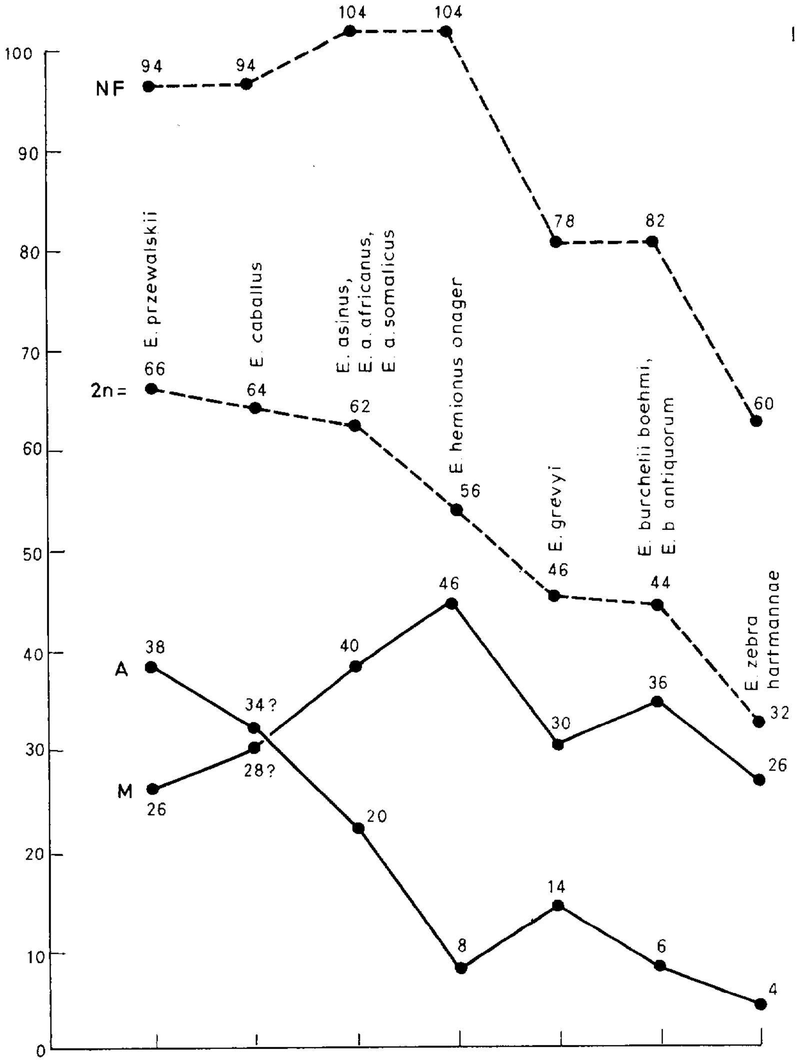

Figure 1 Chromosomes in the Equidae family
2N = diploid number; A = acrocentric autosomes; M = metacentric autosome; NF = nombre fondamental (Matthey) = number of principal chromosome arms

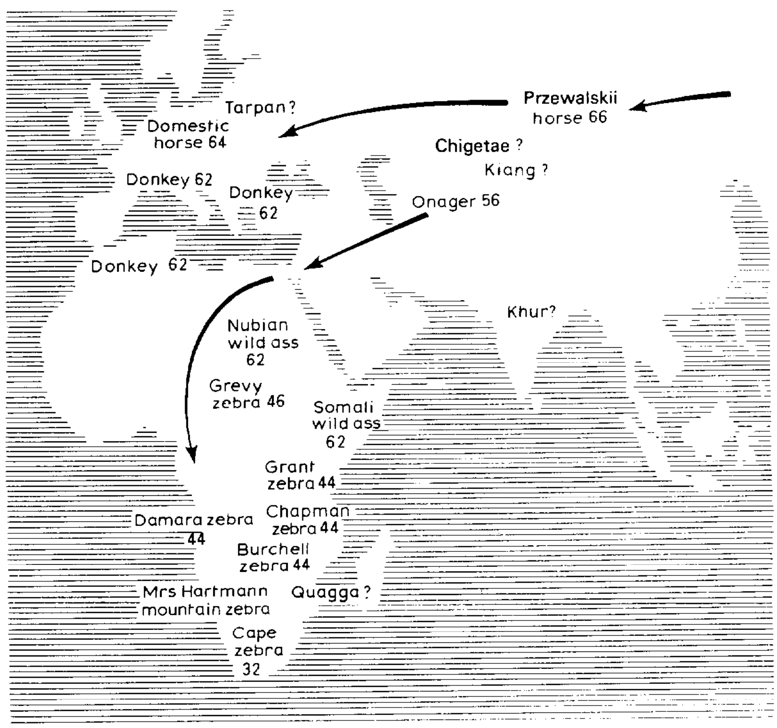

Figure 2 Migratory path (arrows) during evolution of horses, with principal recent members and their chromosome numbers. Chigetae, Kiang, and Khur (three hemiones) have not been studied. Tarpan and Quagga are now extinct